Part I

Partial Purification and Preliminary Analyses of Certain Soluble Azoproteins from Livers of Rats Fed Aminoazo Dyes.

by

Sam Sorof, Marilyn G. Ott and Emily Young

Miller and Miller have demonstrated that the livers of rats fed carcinogenic azo dyes or certain seemingly exceptional non-carcinogenic azo dyes contain protein-bound dyes (1,2). However, protein-bound dyes are absent in the resulting induced liver tumor. Price, Miller and Miller (3) found that over one-half of these dyes are bound to soluble liver proteins. Seemingly in parallel with these findings, Sorof, Cohen, Miller and Miller demonstrated that the liver proteins of a small pair of electrophoretic components, h_1 and h_2, contain the bulk of these soluble bound azo dyes (4). In addition, Sorof and Cohen (5) reported that compared to normal, preneoplastic, or non-neoplastic rat liver, the amount of these h-proteins is markedly reduced in dye-induced liver tumors. These and other findings appear to be compatible with the "protein deletion" hypothesis of azo dye carcinogenesis of Miller and Miller (1,2).

Only the final phases of this study (6) were completed during the covered period. This investigation dealt with the electrophoretic resolution of the h-proteins into six very small subclasses; the isolation of two such partially purified h-subfractions; the concentration of protein-bound azo dyes therein; and the absence of significant amounts of either nucleic acid or riboflavin in such electrophoretically isolated and dialyzed h-proteins.

Adult rats of both sexes were fed ad libitum either 0.06% of the parent carcinogen, 4-dimethylaminoazobenzene (DAB), for $4\frac{1}{2}$ to 5 weeks; or 0.058% of the more potent carcinogen, the 3'-methyl derivative (3'-Me-DAB), for 18 to 21 days; or 0.064% of the very weak carcinogen, 4'-Me-DAB, for 11 weeks; or 0.058% of the non-carcinogen, 2-Me-DAB, for 11 weeks incorporated into the 12% casein diet #3 of Miller and Miller (7), containing 1.0 mg. riboflavin/kg. As shown by Miller and Miller (1,2), the liver contains maximum quantities of the corresponding protein-bound dyes resulting from such feedings at these times. Control rats were similarly fed the same diet without dye for equal periods of times.

In the preparation of the soluble proteins of rat liver, all rat livers were perfused with cold homogenizing buffer and then homogenized in a Potter-Elvejhem homogenizer in 0.08 M sodium phosphate, pH 7.8, containing 0.075 M sodium chloride. This homogenate was ultracentrifuged at 115,000 g for one hour. Use of concentrated protein solutions, large electrophoresis cells of long optical depth, together with long optical lever arm have permitted the detection of very small electrophoretic components which have been revealed by the very high separating power of convection barrier electrophoresis, a method originated in our laboratory (8). In this technique, a single sucrose density gradient placed at the bottom of a column of protein solution effectively isolates disrupting convection gradients from the separating protein solution above, overcoming thereby the hitherto practical limit in the electrophoresis of slowly migrating components in natural protein mixtures.

1003541215

Source: https://www.industrydocuments.ucsf.edu/docs/ztpl0000

Of particular practical importance is the finding that chloride ion apparently specifically prevents the irreversible insolubilization of h-proteins during an early stage of electrophoretic fractionation. Hence, almost all buffers coming in contact with h-proteins in our experiments have contained chloride ion.

By these means, the h-proteins have been electrophoretically expanded 27 times that formerly reported (9). Thus, the h_1-proteins have been resolved into two components; the h_2 into three; and the presence shown of another component, h_3, previously hidden in the salt boundary. Relatively minute components have thus been revealed with a very high degree of resolution between components differing by as little as 0.13 of a mobility unit. Hence, for example, the slow-h_2 proteins of the above DAB or 3'-Me-DAB fed rats represent 1.0% of all the liver proteins.

The following two fractions of h-proteins from rats fed the 3'-Me-DAB diet were isolated: (a) the h_3 region, (b) the slow h_2 + middle h_2 region. These were then analyzed electrophoretically in sodium veronal, pH 8.6, 0.1μ Since the descending proteins denature due to the absence of chloride, only ascending patterns were so analyzed. Figure I shows the analyses of isolated h_3-proteins. Only after prolonged electrophoresis does an asymmetry appear, indicative of heterogeneity. However, electrophoreses at lower pH and ultracentrifugal analyses each reveal the presence of three types. Figure II shows similar analyses of the other isolated fraction, consisting of 57% h_3, 30% h_2-slow, and 13% h_2-middle.

Assuming the absence of protein-bound dye with the middle-h_2 proteins, present only as 13% of this fraction, direct protein bound dye analyses indicate in the case of 3'-Me-DAB fed rats that the h_3-proteins, which represent 6.8% of all the soluble proteins of rat liver, contain approximately 12% of the soluble protein-bound dyes. Likewise, the slow-h_2 proteins, which are only 1.9% of all the soluble proteins of these azo dye fed rats contain approximately 24% of all the protein-bound dyes. Similar results have been obtained with the other carcinogens, DAB and 4'-Me-DAB, as well as with the protein-binding non-carcinogen, 2-Me-DAB (see Part II).

Through the original studies of Kensler and co-workers (10), and many thereafter, dietary riboflavin is known to inhibit certain azo carcinogens. In addition, the nucleic acids have been implicated in protein synthesis, growth, and heredity. For these reasons, it appeared of interest to determine whether the isolated soluble h-proteins from azo dye or control fed rats might contain flavo- or nucleoproteins. Since these h-proteins were isolated by electrophoresis and subsequent dialyses in the cold against pH 7.4 buffer, followed by distilled water, possibly the preparative electrophoresis and the dialyses may have removed any flavin or nucleic acid present, if they dissociate under these conditions. However, within this limitation, it can be said that as isolated the h_3- and h_2-slow proteins from control or 3'-Me-DAB fed rats do not contain significant amounts of either riboflavin or nucleic acid. Table I shows that the RNA content analyzed to be only 0.02% and the DNA content found was only 0.07%. Likewise, as presented in Table II, fluorimetric analysis for riboflavin, based on the assumed usually found 1 mole of flavin per 70,000 grams of protein, revealed the extremely small maximum probable flavoprotein content in these isolated h-proteins of less than 0.15% for rats fed 3'-Me-DAB, and 0.02% for their control.

10035412 16

Source: https://www.industrydocuments.ucsf.edu/docs/ztpl0000

In summary, by original techniques the h-proteins, previously found to contain the bulk of soluble azoproteins after azo-carcinogen feeding and found to be sharply reduced in the azo dye-induced tumor, have been electrophoretically resolved into six subclasses. Two partially purified h-subfractions have thus been isolated and shown to contain protein-bound azo dyes. Further, such isolated and dialyzed h-proteins from control or dye fed rats do not contain significant amounts of nucleic acid or riboflavin. This is of interest considering the roles of the nucleic acids in growth and the inhibitory effect of dietary riboflavin on certain azo carcinogens.

10035412 17

Source: https://www.industrydocuments.ucsf.edu/docs/ztpl0000